36. Observations on the Development of the Sympathetic Nervous System and Suprarenal Bodies in the Sparrow.

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(Text-figures 1–20.)

INTRODUCTORY.

In the course of an investigation suggested to us by Prof. J. P. Hill, as to the origin and development of the suprarenal bodies in the Sparrow, we have been led from a consideration of the source of the chromaffin cells in those bodies, to an examination of the development of the sympathetic nervous system. The origin of this system has been a much-debated question for many years, and the excellence of the material provided for us by Prof. Hill has tempted us to put forward an account of our observations on this subject, more particularly as concerns the relations of the so-called primary and secondary sympathetic cords.

While confirming on the whole the view maintained by the majority of modern embryologists as to the ectodermal origin of the sympathetic system, the investigation has brought to light certain interesting facts which do not appear to have been recorded by previous observers, while we venture to think that the confirmation of previously recorded observations may be of some value.

Our observations have been made on a series of nearly a hundred Sparrow embryos collected during the years 1919–21. Unfortunately little is known as to the exact age of these embryos, but by a careful comparison of the stages of development reached we have been able to arrange them in a series. Keibel's 'Normal Table of Chick Embryos' has been of considerable assistance in this task.

The embryos were fixed by various methods, the most successful being that of Gerhardt. Prof. Hill also placed at our disposal some chick embryos treated by Bielschowsky's method. The best staining results were obtained by the use of Heidenhain's iron hæmatoxylin.

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* Communicated by Prof. D. M. S. WATSON, F.R.S., F.Z.S.

As excellent historical reviews of this subject can be found in the papers of Kuntz (**11**), Goormaghtigh (**6**), and Da Costa (**3**), and in the 'Text-Book of Human Embryology' of Keibel and Mall, we do not attempt a further summary, but confine ourselves to a restatement of previous observations on the primary and secondary sympathetic chains in birds and in such other vertebrates as have been observed to possess them.

Wm. His, Jun. (**8**), was the first to describe the occurrence in chick embryos of primary and secondary sympathetic chains. He observed, in a 4-day chick, a sympathetic chain similar to that found in other vertebrates, *i. e.* situated immediately behind (*i. e.* dorsal to) the carotids and on each side of the dorsal aorta. At the 8th day, however, he found a second cord immediately in front of the anterior (ventral) roots of the spinal nerves. This second cord, he says, soon outgrows the former in size and is enclosed in the vertebrarterial canal. He was unable to trace the subsequent fate of the primary cord.

In a later paper (**9**) the same author again discusses the relations between the primary and secondary cords. He is of opinion that the primary cord in the neck region atrophies, whilst in the thoracic and abdominal regions it forms the aortic plexus and thence migrates along the mesentery to the organs situated in those regions. He regards the primary sympathetic of the trunk region as only a step on the path of the sympathetic cells from the spinal ganglia to the periphery, and lays considerable stress upon the resistance offered to the migratory nerve cells by the mesodermal elements.

The secondary sympathetic cords he describes as the latest formation of the spinal ganglia, and suggests that the formation of the former is due either to a loss of migratory power in the sympathetic cells or to an increased resistance offered by the mesoblast.

Miss W. Abel (**1**) adopts the views put forward by His, Jun., but has observed a small contribution from the primary to the secondary sympathetic cord, although she lays little stress on her important observation.

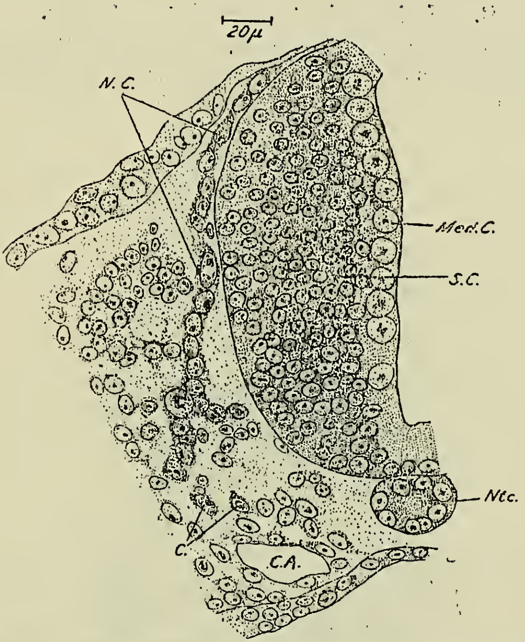
Kuntz (**11**) and Ganfini (**5**) support the main findings of His. They have also observed in Chelonia the existence of a secondary sympathetic. All these writers are agreed in describing the secondary sympathetic as a new formation quite independent from the primary sympathetic system.

A. *Relations of the Sympathetic Primordium to the Neural Crest.*

While Held (**7**) derives the sympathetic primordia exclusively from the dorsal root ganglia, and Kuntz (**12**) is of opinion that the chief part is derived by way of the ventral roots, our observations lead us to support the view put forward by Streeter (**12**). He writes: "As the neural crest becomes detached and its segmenting part invades the space between the myotomes and the neural tube, certain ganglion cells separate themselves from its ventral border and independently migrate ventralward into the neighbourhood of the aorta. It is these cells that form the connected chain of errant ganglia which we know as the sympathetic system."

Thus in Sparrow 16 of 6–7 somites [= 32-hr. chick] we find a well-marked neural crest, reaching from the dorsal border of the spinal cord nearly to its ventral edge. From the distal end of the crest, cells appear to be proliferated into the mesenchyme between spinal cord and aorta (text-fig. 1). The crest at first consists, in section, of a single row of cells with deeply staining cytoplasm and with large, oval, lightly staining nuclei. They have usually one large nucleolus, but two are sometimes present. The cells of the crest show mitotic figures especially at its ventral edge. Between the latter and the dorso-lateral angle of the

Text-figure 1.



Transverse section of Sparrow 16 (6–7 somites), showing spinal cord and developing neural crest. From the ventral end of latter cells (c) are proliferated towards the carotid artery.

C. Migrating cells. *C.A.* Carotid artery. *Med.C.* Ependymal cells. *N.C.* Neural crest. *Ntc.* Notochord. *S.C.* Spinal cord.

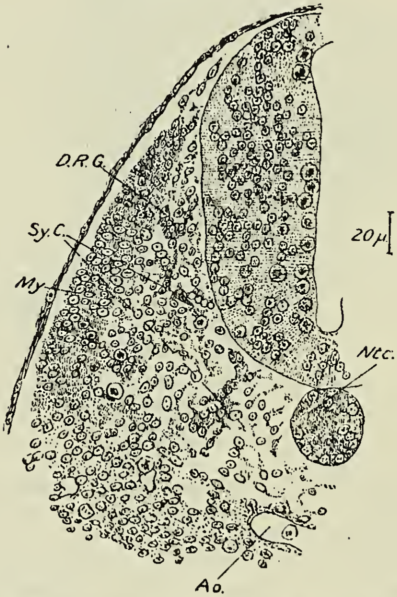
(Sp. 16, slide 3, row 3, section 12.)

aorta there are isolated cells with identically the same characteristics as those of the crest, and clearly distinguishable from the surrounding mesenchyme cells. The latter have much smaller and spherical nuclei and are distinctly branched, their processes being united so as to form a cell network. Their cytoplasm stains much lighter than that of the crest cells. The cells proliferated from the neural crest are not found in every section, but are most marked in the inter-somitic regions, where later we find the ganglia of the primary sympathetic cords.

B. *Relations with the Dorsal Root Ganglia.*

With the expansion of the neural crest to form the dorsal root ganglia the process of proliferation of sympathetic cells increases, and as is shown in text-fig. 2 from Sparrow 15 (= 42-hr. chick)

Text-figure 2.

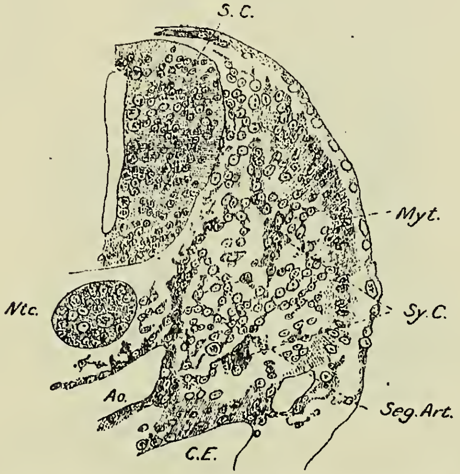


Transverse section of Sparrow embryo 15 (= 42-hr. chick), showing a succession of cells (*Sy.C.*) migrating to the dorso-lateral angle of the aorta.

Ao. Aorta. *Sy.C.* Sympathetic cells. *D.R.G.* Dorsal root ganglia. *My.* Myotome. *Ntc.* Notochord. (Sp. 15, 2, 4, 15.)

there is a succession of cells apparently migrating to the dorsolateral angle of the aorta. These cells occur between the myotome, now considerably developed, and the spinal cord. The cells still possess large clear oval nuclei above described, but the surrounding cytoplasm appears to lose its individuality, with the resulting formation of syncytial masses or strands, in which there is evident mitotic activity. Kohn (12) figures such strands in the Rabbit. Streeter (12) considers this syncytial character to be only transitory, and associates it with the period of migration. Contemporaneously with this migratory activity of nervous elements the primordia of the segmental arteries and veins are being laid down. Thus in text-fig. 3 we see that the endothelium of the dorso-lateral angle of the aorta has proliferated to form a distinct strand of cells, which will ultimately develop into a segmental artery. Similar vascular strands may be seen in other sections arising from the cardinal veins. The nuclei of the cells in these vascular strands are very irregular in shape, and their cytoplasm is considerably lighter and less granular than that of the nerve strands. In view of the fact that some authors have been led to consider such strands as of nervous significance, we venture to direct special attention to this differentiation.

Text-figure 3.



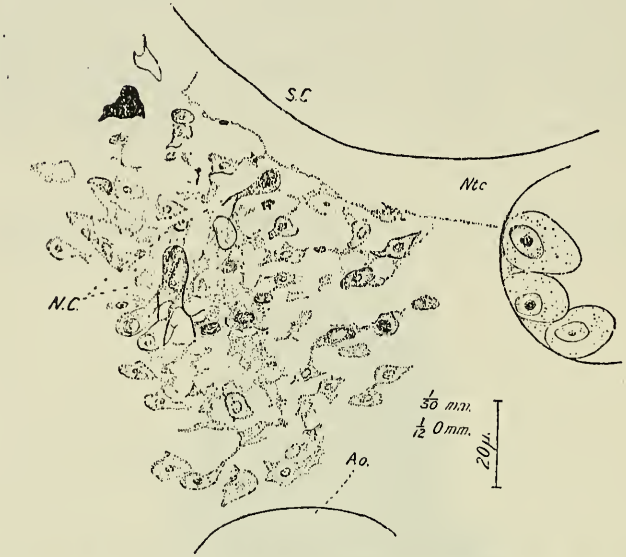
Transverse section of Sparrow embryo 15 (= 42-hr. chick), showing development of segmental artery and migration of sympathetic cells (*Sy.C.*).

Ao. Aorta. *C.E.* Cœlomic epithelium. *Myt.* Myotome. *S.C.* Spinal cord. *Seg.Art.* Segmental artery. *Sy.C.* Migrating sympathetic cells. *Ntc.* Notochord. (Sp. 15, 3, 1, 1.)

That the cells migrating from the dorsal ganglia are neuroblasts is confirmed by silver preparations of chick embryos in which the ventral roots have not yet appeared, *i.e.* of less than

60 hrs. Text-fig. 4 is drawn from a section of such an embryo. It shows neuroblasts isolated between spinal cord and aorta possessing distinct nervous processes. The neuroblast has a large oval nucleus, and its processes are directed towards the aorta.

Text-figure 4.



Transverse section of chick of $2\frac{1}{2}$ days (Bielschowsky preparation), showing amid the mesenchyme cells isolated neuroblasts (*N.C.*) between spinal cord (*S.C.*) and aorta (*Ao.*).

C. *Relations with the Ventral Roots.*

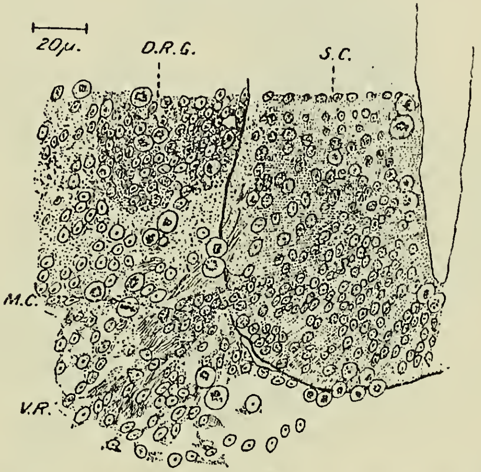
Whilst all investigators are not agreed as to the origin of sympathetic cells from the neural crest before its differentiation into the dorsal root ganglia, most are agreed that the ganglia when formed give origin to the sympathetic cells. Held (**7**) goes so far as to derive the whole of the sympathetic ganglia from the *dorsal* root ganglia. His evidence is based on the examination of Selachian material, but he is so satisfied with those observations that he applies his deductions to the case of chick embryos, although he confesses that he has not been able to discriminate the roots satisfactorily in them.

Kuntz (**11, 12**), on the other hand, attaches by far the greater importance to the ventral roots as the path by which the cells migrate, a view which he supports by both morphological and experimental evidence.

In collaboration with O. V. Batson he experimented on chick embryos. By electrolysis they destroyed the dorsal part of the neural tube of the embryos, and in four successful cases found that the sympathetic primordia were formed to a greater or less extent. Kuntz later carried out further work on the same lines and arrived at similar results. Of similar experiments carried out on tadpoles, by actual dissection, he writes: "As far as the study of the development of the sympathetic trunks was pursued in these embryos the findings corroborated those in the operated embryos of the chick."

The experimental investigations of E. Müller and Sven Ingvar (**13**), on the other hand, support the conclusions of His and Held.

Text-figure 5.



Transverse section of Sparrow embryo 11 (= chick about 78 hrs.), showing cells leaving the spinal cord by the ventral nerve root.

D.R.G. Dorsal root ganglion. *M.C.* Migrating cells. *S.C.* Spinal cord. *V.R.* Ventral root. (Sp. 11, 4, 2, 17.)

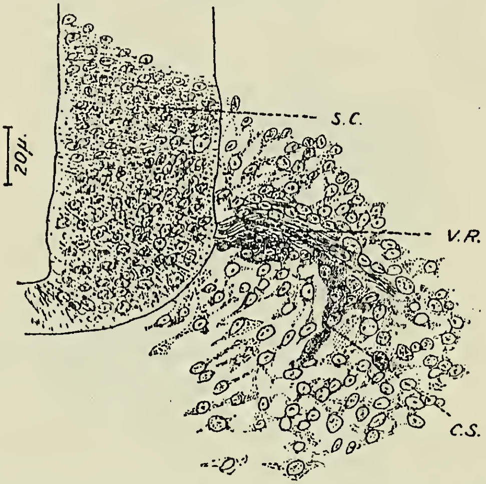
They carried out their experiments on Amphibian material. By destroying the ventral part of the neural tube they found that the sympathetic was still formed, while the removal of the dorsal portion resulted in its failure to develop. They thence conclude that the primordia of the sympathetic nervous system arise from the spinal ganglia.

The conflicting results obtained by these workers illustrate the pitfalls inherent in the experimental method as applied to this particular problem.

Our observations certainly do not support Held's view as to the exclusive origin of the sympathetic system from the dorsal root ganglia, but they lead us to attach somewhat more importance to that source of origin than Kuntz would seem to allow.

Text-fig. 5 shows the ventral root of a Sparrow embryo, No. 11 (= 78-hr. chick). The ventral roots are well developed, and the one figured shows a group of cells close to the origin of the root, and evidently in course of migration. That the migration is outwards seems clear from the observation of a later stage, such as is reproduced in text-fig. 6 of Sparrow 51 (= 84-hr. chick). Here we find some of these migrating cells leaving the ventral root and making their way to the dorso-lateral angle of the aorta, at which point they join the previously described sympathetic cells, and with them form diffuse masses of cells, which are the primordia of the primary sympathetic chains. At this stage the chains are not formed, but the diffuse cell masses occur metamerically at intervals corresponding with the somites.

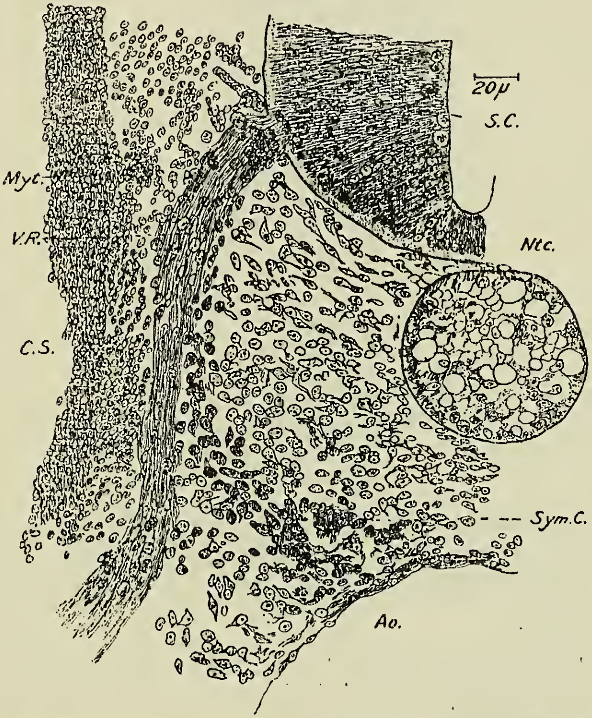
Text-figure 6.



Transverse section of Sparrow 51 (= 84-hr. chick), showing cells leaving ventral root in the direction of the dorso-lateral angle of the aorta.
C.S. Cell strand. *S.C.* Spinal cord. *V.R.* Ventral root.
(Sp. 51, 3, 2, 4.)

This migration of cells along the ventral roots, and thence to the dorso-lateral angle of the aorta, is visible to a greater or less extent in all the embryos examined at about this stage (= 3–3½-day chick). Owing to the curvature of the embryo it is obvious that the occurrence of a transverse section passing along one of these migratory paths is quite fortuitous, though it can always be shown by reconstructions from consecutive sections, a method which Held employed for some of his illustrations. Text-fig. 7, however, shows a fortunate section of a chick embryo (D. 4 of 3½ days), in which the curved track of darker cells is practically continuous, thus furnishing a striking picture of the path of migration. This path seems to be markedly affected by the segmental arteries and veins, for it is between these vessels that the sympathetic cells move to their primary destination (see text-fig. 3). It is possible that the intersegmental region offers a path of least resistance to this migration.

Text-figure 7.

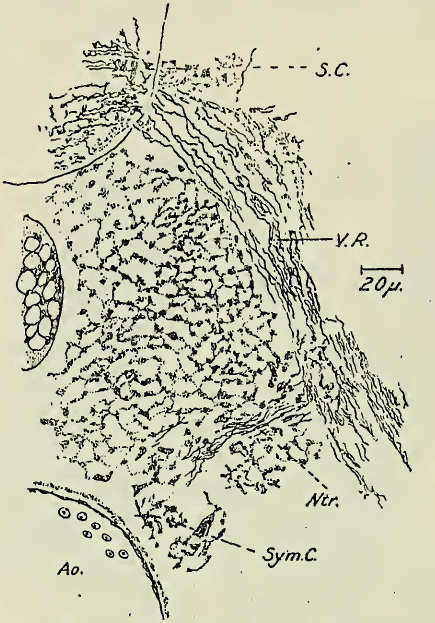


Transverse section of chick embryo (3½ days, D 4), showing an almost continuous strand of sympathetic nerve cells from the point of exit of the ventral nerve root to the dorso-lateral angle of aorta.

Ao. Aorta. *C.S.* Sympathetic cell strand. *Ntc.* Notochord. *Myt.* Myotome. *S.C.* Spinal cord. *Sym.C.* Group of sympathetic cells. *V.R.* Ventral root.

A track of nerve fibres in the same position is clearly marked in silver preparations, such as that shown in text-fig. 8, and it seems probable that this path would agree with that of the migrating nerve cells.

Text-figure 8.



Transverse section of chick (m–4½) from a Bielschowsky preparation, showing nerve fibrils pursuing the same course as the sympathetic cell strand in fig. 7. The mesenchyme and other cells are much distorted, but some of those near the dorso-lateral angle of the aorta show evidence of being sympathetic in character.

Ao. Aorta. *S.C.* Spinal cord. *V.R.* Ventral root fibres. *Sym.C.* Sympathetic cells. *Ntc.* Notochord. *Ntr.* Nerve fibres in the track of migrating sympathetic cells.

While it appears that many of these cells are sympathetic in nature, the possibility must not be excluded that some of them will develop into the sheath cells of Schwann.

D. *The Primary Sympathetic Chain.*

In their earliest stage the primordia of the sympathetic chains consist of two parallel series of cell groups situated at the dorso-lateral angles of the aorta. These are arranged metamerically and show a more advanced stage of development in the anterior thoracic region. Ensuing stages show their gradual consolidation by the dispersed cells gathering into compact masses, and the simultaneous growth of longitudinal cellular strands, which unite them into continuous moniliform chains. Silver preparations show scattered fibres among these cells. A graphic reconstruction of the consolidated cord in the anterior thoracic region at this stage is shown in text-fig. 9 A.

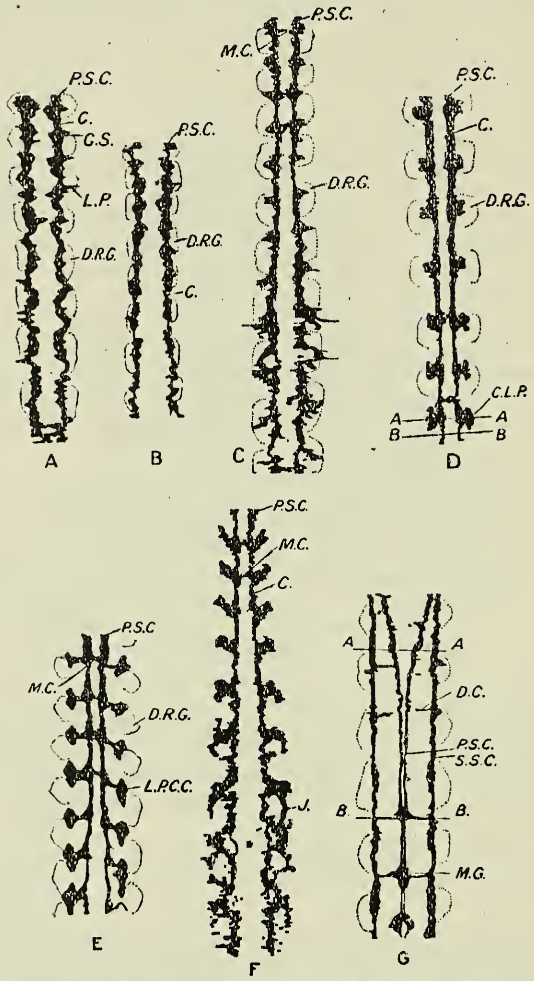
So far as we have been able to observe, the cells constituting the chain at this stage do not show any differentiation of chromaffin cells among them. Their nuclei are oval, their cytoplasm darkly stained and full of minute granules, cell boundaries are faint, or may still be absent. We have constantly observed the presence of clear spaces surrounding the groups of sympathetic cells near the dorso-lateral angle of the aorta. Kuntz has noticed these clear spaces. They may be spaces filled with lymph during life, or they may be due to shrinkage phenomena. We have followed the development of the cords by means of graphic reconstructions from a series of Sparrows, separated by very slight differences of development, and the result is striking. The series of reconstructions is shown in text-fig. 9.

We have chosen for purposes of reconstruction the anterior thoracic region of the embryo, where its development is most advanced. Text-figs. 9 A & B are reconstructions of the primary sympathetic nervous system of Sparrows 7 and 2 (= 78-hr. chick). They show that at this stage the sympathetic chains in the anterior thoracic region consist of two approximately parallel cords, formed by masses of cells united by their connecting strands. Anteriorly the strands are much thicker, but not sufficiently so to hide the metamerism, which corresponds exactly with that of the dorsal root ganglia. In both the text-figures, but more markedly in text-fig. 9 B, it will be seen that the swollen parts of the cord send out lateral processes. These processes do not run in the plane of the parallel cords, but are directed dorso-laterally. The apparent differences in the directions to which they point are due to the curvature of the embryo.

Text-fig. 9 C is from Sparrow 6 (= 84-hr. chick). The moniliform character of the strands is more marked and gives the impression that the swellings have increased at the expense of the connectives. The distances between the ganglionic swellings, *e. g.* in text-figs. 9 A & 9 C, are roughly equal, but the connectives in the latter have become more slender, a result which could be attained either by a process of degeneration, which seems improbable at this early stage, and of which no evidence was obtained in the sections, or by migration of their elements into the ganglionic swellings.

Similar migration seems to have taken place into the lateral processes, which are now connected by broad bases to the primary ganglia, and taper off to a point dorso-laterally to the cords. This certainly suggests that their growth is outward from these ganglia, a view supported by the frequent occurrence of mitoting

Text-figure 9.



Graphic reconstruction of the sympathetic primordia in the anterior thoracic region of Sparrows. The cord is viewed from the ventral surface and the metamerically arranged dorsal root ganglia are indicated by dotted lines. The lateral projections are not in the same plane as the cords, and allowance must be made for the necessary foreshortening in their projection.

A. Sparrow 7 (= 78-hr. chick). Cords continuous with slight swellings and short dorso-lateral processes.

B. Sparrow 2 (= 78-hr. chick). Cords continuous with slight swellings and short dorso-lateral processes.

C. Sparrow 6 (= 84-hr. chick). Swellings and processes much more pronounced.

Notice four mesial commissures and the proliferation at the posterior end which is the region where there exists a close relation between the sympathetic and the suprarenal bodies.

cells in both ganglia and processes. The posterior part of the chain in text-fig. 9 C still shows considerable dispersion of the sympathetic elements, and it is from this region that contributions are sent to the suprarenal bodies. In the anterior part the chains are united by transverse commissures.

Text-fig. 9 D represents a reconstruction of Sparrow 13 (= 96-hr. chick). The distinction between ganglia and connectives is much more marked, and an important change is also visible in the lateral processes. The four anterior pairs are still united by a broad base to the primary ganglia, but instead of tapering to a point they are now of practically the same thickness throughout. In the three posterior pairs the bases show signs of constriction, while the distal portion is marked by a cranio-caudal elongation. Now when we examine the cords themselves we find that they are thinnest just in the region where the lateral processes possess these cranio-caudal extensions, while in the anterior region, where the processes have not yet reached this stage, the cords remain of considerable thickness. We are again driven to the conclusion that the cranio-caudal extensions are formed at the expense of the primary cords.

Text-fig. 9 E, from Sparrow A (= 5½-day chick), shows a continuation of this development. The lateral processes are now solid masses, united to the primary chain by short connectives, and all of them show cranio-caudal extensions, the caudal being more marked. The correlation between the increased size of the lateral process and the thinness of the primary sympathetic chain is again very obvious.

The solid lateral masses are now situated in close relationship with the spinal nerves, being located just mesial to the junction of dorsal and ventral nerve roots. They may now be termed the primordia of the secondary sympathetic ganglia. Subsequent growth of their cranio-caudal extensions leads by their union to the formation of the definitive sympathetic chain (secondary sympathetic chain).

D. Reconstruction of Sparrow 13 (= 96-hr. chick). Ganglia and connectives more marked. Lateral processes thickened. Posterior ones constricted to some extent.

E. Reconstruction of Sparrow A (5½-day chick). Lateral processes now solid masses united to the primary chain by short connectives. All show cranio-caudal extensions.

F. Reconstruction of Sparrow 60 (= 6-day chick). Cranial-caudal extensions meet to form secondary sympathetic chain.

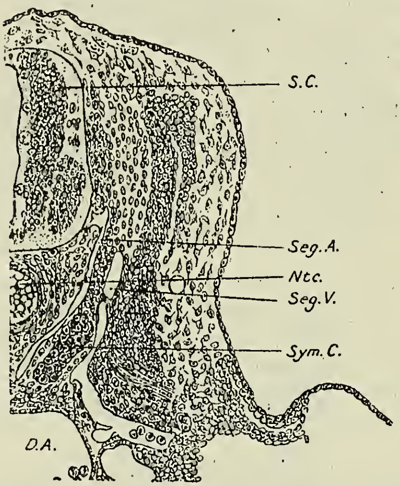
G. Reconstruction of Sparrow B (= older than Sparrow 60). Secondary sympathetic chain formed. Connectives between primary and secondary degenerating.

C. Connective. *D.R.G.* Position of dorsal root ganglion. *G.S.* Ganglionic swelling. *P.S.C.* Primary sympathetic cord. *M.C.* Median connective. *C.L.P.* Constricted lateral process. *L.P.C.C.* Lateral processes with cranio-caudal extensions. *J.* Junction of cranio-caudal extensions. *S.S.C.* Secondary sympathetic chain. *M.G.* Median ganglia. *D.C.* Degenerating connective.

E. *The Secondary Sympathetic Nerve Cords.*

An interesting example of this union is shown in text.-fig. 9 F, a reconstruction of Sparrow 60 (= about 6-day chick). We here see that at two points the secondary chain is actually completed, while in other places marked caudal prolongations are apparent. The completion of the formation of these definitive sympathetic chains is found at a stage corresponding to that of the seven-day chick. A reconstruction of this stage is shown in text-fig. 9 G, Sparrow B.

a. Text-figure 10 (*a* & *b*). *b.*



Diagrammatic figures showing relations of sympathetic cord to surrounding tissues as seen in transverse sections of Sparrow 13 at points marked A–A, B–B in fig. 9 D.

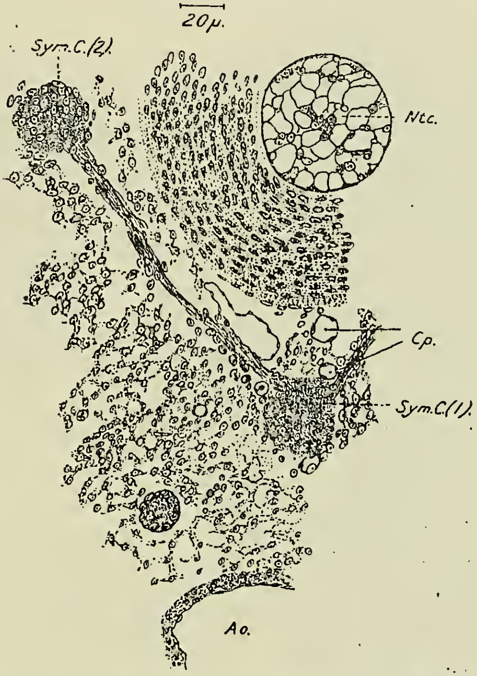
10 *a*. Passes through a ganglionic swelling of the sympathetic chain (*Sym.C.*). The ganglionic swelling lies dorso-lateral to the aorta (*D.A.*) and between the segmental artery (*Seg.A.*) and segmental vein (*Seg. V.*). There is a dorso-lateral projection pointing towards the position of the dorsal root ganglion.

10 *b*. Passes through a connective (*P.S.C.*) between two ganglionic swellings. The two sections are only 56 μ apart.

D.A. Dorsal aorta. *D.R.G.* Dorsal root ganglion. *Myt.* Myotome. *Ntc.* Notochord. *Sp N.* Spinal nerve.

The condition of the primary sympathetic chain at this stage presents very interesting features. The two cords are now much diminished in size and, gradually approaching one another, are for some distance almost in contact. Their moniliform character is lost, except that in the thoracic region we find three large median ganglia, partly or completely fused (text-fig. 11). These are joined to the secondary ganglia by connectives of nervous fibres. We have also found these median ganglia in a much more advanced embryo. The ganglia lie above the aorta immediately ventral to the vertebral column, the centra of which are in process of development. On either side of them there occur mesodermal masses, such as give rise to muscle in the adult, in this case probably to muscles attached to the vertebræ. The connectives between the ganglia are associated with a rich capillary plexus dorsal to the aorta.

Text-figure 11.



Transverse section through Sparrow B at point marked A–A in fig. 9 G. The secondary sympathetic ganglion is shown at *Sym.C.* (2) and is connected with the median fused ganglia of the primary sympathetic (*Sym.C.*) (1) chain.

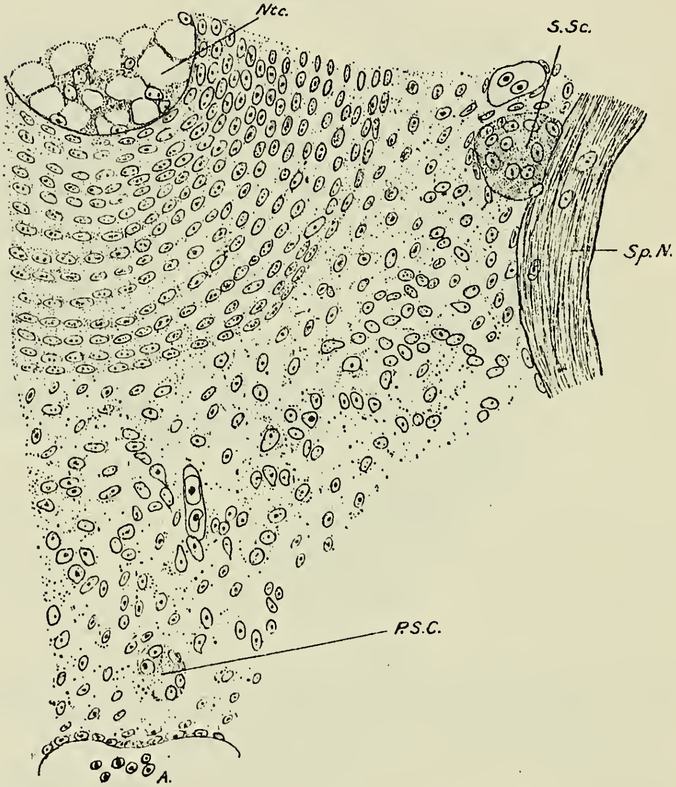
Ao. Aorta. *Cp.* Capillaries associated with primary sympathetic.

(Sp. B 9, 4, 7 and 8.)

Posterior to these ganglia the primary sympathetic cords diminish in size, until in the abdominal region it is no longer possible to trace them.

Transverse sections throw light on the processes of development above described. Text-figs. 10 *a* & 10 *b* show transverse sections of Sparrow 13 at the points marked A–A, B–B in text-fig. 9 D. The sections are only 56 μ apart, but the difference is very marked. The section shown in text-fig. 10 *a* passes through the dorso-lateral processes of the primary sympathetic chain, and shows their relations to the surrounding structures. They lie between the segmental artery and vein, and extend dorsolaterally from the aorta to a point about halfway towards the origin of the ventral roots. The section in text-fig. 10 *b*, on the other hand, shows the primary sympathetic as a comparatively small strand close to the aorta. This section passes through the myotome and the dorsal root ganglion, while that shown in text-fig. 10 *a* passes between the somites.

Text-figure 12.



Transverse section through Sparrow B at point marked B–B in fig. 9 G. The secondary sympathetic chain (*S.Sc.*) is closely apposed to the spinal nerve.

Text-figs. 11 & 12 drawn from sections of Sparrow B, at points marked A–A and B–B in text-fig. 9 G call for some remark. Text-fig. 12 shows the primary and secondary sympathetic chains apparently quite disconnected. The primary has its usual position at the dorso-lateral angle of the aorta, the secondary is situated close against the spinal nerve, and lateral to the notochord. Text-fig. 11, however, shows a section through the connective between primary and secondary chains. The connective at this stage is composed chiefly of nerve fibres, but along them there are scattered cells, which are probably destined to form sheath cells. The two primary ganglia in this section will be seen to have completely fused.

F. *Rami Communicantes.*

The foregoing description of the development of the sympathetic nervous system will explain how there has arisen a considerable amount of confusion as to the *rami communicantes* in birds, for it will be seen that in this group there are no less than three distinct sets of connections associated with the central nervous system and the outlying sympathetic. These are :—

1. The strands of nerve cells which give rise to the primary sympathetic cords.
2. The constrictions of the dorso-lateral processes by which the primary cords are attached to the secondary ganglia.
3. The definite connections by which the secondary ganglia become attached to the spinal nerves.

Strictly speaking the term *ramus communicans* should be restricted to the last of these three groups, but previous papers and illustrations show that all these three forms have been thus designated. Ganfini (5) goes so far as to use the terms primary, secondary, and tertiary *rami communicantes* for the three classes of connections, but in view of their significance in neurology it would seem preferable to apply the name only to those fibres by which the definitive sympathetic is linked up with the mixed spinal nerve. As the definitive *ramus* does not arise till about the stage corresponding to that of a seven-day chick, it is evident that any connection between the sympathetic and the central nervous system, existing in earlier stages, must be looked upon as an example of either the first, or the second, of the types of connections above mentioned. As the history of their development shows, the connections of groups 1 and 2 have only a transient existence, with the possible exception of the connections in certain plexuses.

The three types of connections are characterized more or less definitely by their morphology. Those of the first group are formed of discontinuous cells, though silver preparations show that scattered nerve fibres occur in them. Connections of the second type, on the other hand, are formed by compact masses of cells, especially at their first appearance. In the course of their development they become elongated and may be narrowed down almost to a single row of cells. In their final stages their cellular character disappears by migration of the cells to the secondary chain, and a fibrous connection is left (text-fig. 11). With the exception of certain connections which are maintained with the chief plexuses of the trunk, these also ultimately disappear, usually after the appearance of the definitive *rami*. These last are from the beginning purely fibrous. Their development consists merely of a growth in length, as the secondary sympathetic ganglion moves away from its early position at the point of union of dorsal and ventral roots. The range of movement varies in different regions, being very small in the neck. Thébault (14) figures the *rami communicantes* of *Corvus* as sometimes quite distinct in the neck region, but also describes a fusion of the sympathetic ganglion with the spinal nerve, and remarks that all the intermediate stages may be found. A *ramus communicans* can always be found in our material by microscopic examination.

That the sympathetic ganglion, while making its own contribution to the mixed spinal nerves, carries with it fibres from both roots, has been abundantly proved by previous observers. Rossi (16), in his recent paper on the afferent paths of the sympathetic system, demonstrates by direct anatomical observations, that cells occur in the dorsal root ganglia which send their processes into the *rami communicantes*. These he regards as the cells of origin of Kölliker's fibres, and considers them to be afferent sensory neurones.

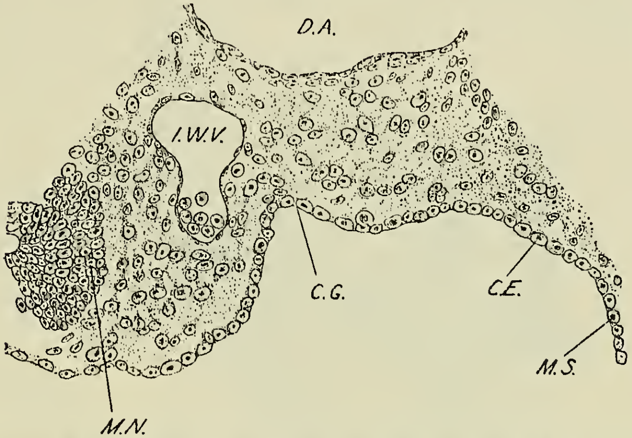
G. *The Suprarenal Bodies.*

Our observations lead us to agree with those who ascribe to these bodies a dual origin: *e. g.* Balfour, Poll, Mitsukuri, Giacomini, and Goormaghtigh.

In Sparrow 7 (= 78-hr. chick), text-fig. 13, there are already signs of the grooves in the cœlomic epithelium from which the cortical portion of the suprarenal bodies will arise. These grooves are situated on either side of the mesentery and close to it. Laterally to them will be found the internal Wolffian vein and the mesonephros, and in certain cases the external glomerulus of the pronephros can also be seen. In text-fig. 14 these relations are clearly visible, and the proliferation of cells has already started at the region described. The cells of the cœlomic epithelium are cubical with large nuclei. Mitotic figures are numerous and the products of division appear to be moving inwards. In Sparrow 8, text-fig. 15, the proliferation of cells becomes more marked, and a ridge is formed internally. These cortical primordia appear to be metamerically arranged. In Sparrow 11, text-fig. 16, the cortical primordia are very clearly marked, but still closely attached by a broad base to the cœlomic epithelium. Groups of cells appear to be moving towards their later position between the aorta and the mesonephros.

Sparrow 5, text-fig. 17, shows the separation completed; the primordia are now compact and contain a few cells from the primary sympathetic chain, which have migrated down the sides of the aorta. It is this contribution from the sympathetic chain which eventually forms the medullary tissue of the suprarenal body.

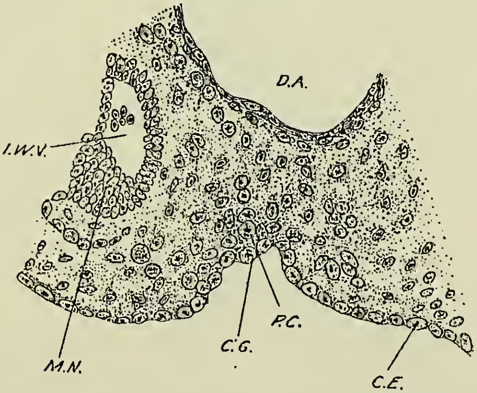
Text-figure 13.



Transverse section through Sparrow 7 (= 78-hr. chick), showing the formation of the cœlomic groove (*C.G.*).

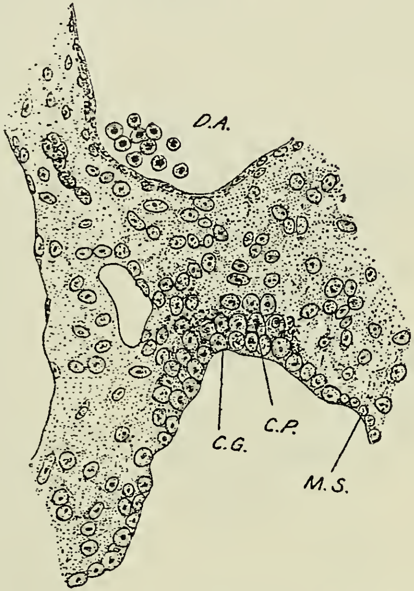
C.E. Cœlomic epithelium. *D.A.* Dorsal aorta. *M.N.* Mesonephros. *M.S.* Mesentery. *I.W.V.* Internal Wolffian vein. (Sp. 7, 5, 3, 3.)

Text-figure 14.



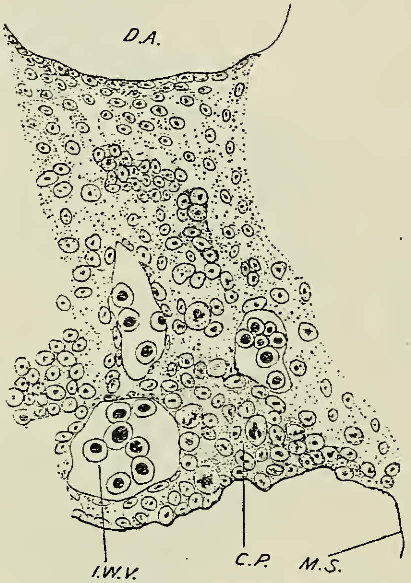
Transverse section of Sparrow 8, showing the deepening of the groove (*C.G.*) and proliferation of cells (*P.C.*) to form the primordium of the suprarenal cortex. Letters as in fig. 13.

Text-figure 15.



Transverse section of Sparrow 8, showing grouping of cells to form the primordium of the suprarenal cortex (*C.P.*). Letters as in fig. 13.

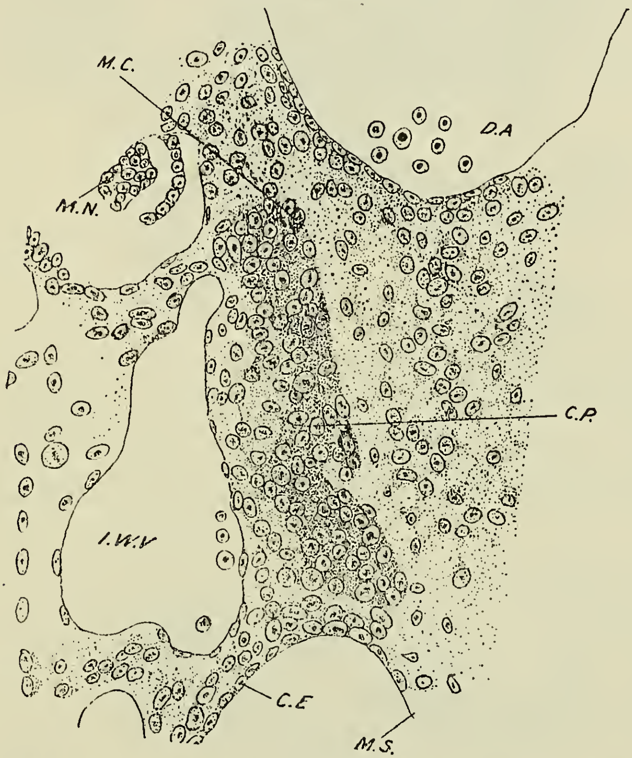
Text-figure 16.



Transverse section of Sparrow 11, showing a further development of the cortex. The suprarenal is now invading the internal Wolffian vein. Letters as in fig. 13.

Text-figs. 18 & 19, from Sparrow 53, show the relations of the sympathetic and the suprarenal body. The latter now surrounds the internal Wolffian vein, while the sympathetic chains dorso-lateral to the aorta are largely developed, and give rise to masses of cells which pass from them to the cortical primordia. The point of entry of these cells seems usually to be on the mesial face of the suprarenal body (text-fig. 18), but in text-fig. 19 there is apparently a dorsal entry.

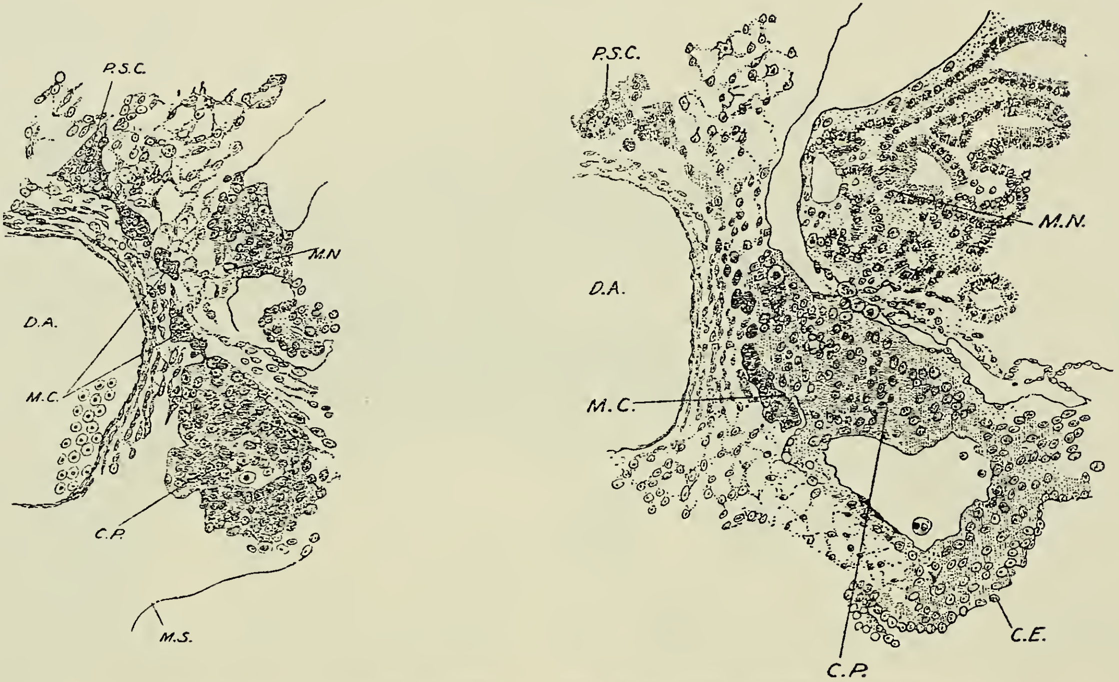
Text-figure 17.



Transverse section of Sparrow 5, showing first signs of invasion of cortex by medullary cells (*M.C.*) from the primary sympathetic. Lettering as in text-fig. 13.

It is noteworthy that these contributions of the sympathetic to the suprarenal all arise from the primary sympathetic cords, which apparently proliferate them at about the same time as they are giving rise to the definitive cords, by the outgrowth of dorso-lateral branches, as described above. We have not attempted a detailed description of the changes which occur in the development of the suprarenal bodies, as these have been

Text-figures 18 & 19.



Transverse sections of Sparrow 53, showing contributions of primary sympathetic to suprarenal body. In fig. 18 the contribution is dorsal while in fig. 19 it is mesial. (Sp. 53, 4, 4, 3. Sp. 53, 4, 5, 4.)

admirably set forth by Goormaghtigh in his recent paper. The only point on which our observations lead us to differ from him is as to the origin of the medullary substance. This is derived, according to our observations, from the primary sympathetic cords and not, as Goormaghtigh maintains, from the mesodermal cells of the sclerotome.

II. *Discussion.*

Since Francis Balfour (2) in 1877 described the development of the sympathetic nervous system in the Selachii, many theories have been put forward supporting, or refuting, his statement as to its origin. Without going deeply into the history of the controversy, we may summarize the chief suggestions as follows:—

A. *Origin ectodermal.*
  a. From the medullary tube.
  b. From the spinal nerve roots.
  c. From the dorsal root ganglia only.
  d. From ventral roots chiefly.
B. *Origin mesodermal.*
  Differentiation *in situ.*
C. *Mixed origin.*
  From the medullary tube and sclerotome.

Supporters of the mesodermal origin of the sympathetic nervous system have based their conclusions chiefly on the presence of the sympathetic primordia, at the dorso-lateral angles of the aorta and the carotids, *before* the appearance of the nerve roots. Although we agree with them in this observation, we are yet of the opinion that the cells in question have not differentiated *in situ*, but that they have been proliferated from the neural crest (see text-fig. 1).

We have not found a trace of support for the interpretation recently put forward by Dart and Shellshear (4), who state that "a mesodermal origin of these neuroblasts (*i. e.* neuroblasts in relationship with mesodermal structures) must therefore be postulated and is demonstrable." All nerve fibres which we have observed could either be traced from the central nervous system, or, in certain cases, from free cells closely associated with it, and in no case occupying a position such as would support their view.

With regard to Goormaghtigh's (6) contention for a mixed origin, we may state that we have kept a careful look out for the strands, which he describes as arising from the sclerotome and contributing to the formation of the primary sympathetic system, and have been entirely unsuccessful in our search. The paths of the migrating cells destined to form the sympathetic primordia certainly pass close to the sclerotomes, but the boundary between the two structures in our material has always been clearly marked, and the cells on either side of that boundary sufficiently differentiated to distinguish them. It is unfortunate that the photographic illustrations, which Goormaghtigh puts forward in support of his view, are far too indistinct to serve as evidence for it.

We are thus led to support the entirely ectodermal origin of the sympathetic nervous system, and from the observations already given, it will be seen that we do not confine that origin to any one part of the central nervous system. The statement by experimental physiologists that the sympathetic contains both afferent and efferent components, while it does not afford a proof of a diverse origin, yet supports the possibility of such being the case. We have seen that some cells originate very early from the neural crest, and we are convinced that both dorsal and ventral roots contribute towards the formation of the sympathetic primordia.

The observations of His (8) and Held (7) support the origin from the dorsal root ganglia. These writers describe the primordia as arising by cells, migrating from the spinal ganglia by way of the spinal nerve, from which they diverge a little beyond the point of their junction, and thence make their way to the dorso-lateral angle of the aorta. The sympathetic cells thus leave the spinal nerves at a point subsequent to the union of dorsal and ventral roots, and so may originate from either of those sources. The assumption that all have arisen from the dorsal root ganglion is based simply on an analogy with Selachian material, and has no foundation on observations of avian embryos.

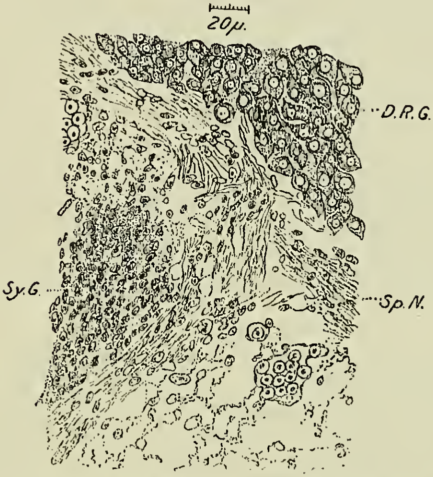
The descriptions of Held and His lead one to the supposition that this process is continuous throughout the development of the sympathetic; this, however, is quite contrary to the results of our investigations. If the sympathetic ganglia were derived directly from the spinal nerve, one would naturally expect the cells composing them to bear a distinct resemblance to those from which they are said to originate; but this we do not find to be the case. The cytological characters of the dorsal root ganglia, and of the cells of the ventral horn of grey matter, are so different from those of both primary and secondary sympathetic ganglia, that there seems no possibility of one being derived immediately from the other (see text-fig. 20).

Our description, as given in § E, of the morphological development of the sympathetic, allows ample opportunity for this differentiation to arise; for we discover that though the ganglia of the dorsal roots and the definitive sympathetic ganglia lie close to one another, yet the latter have had opportunity to undergo considerable changes in the course of their extensive migration, *first*, from the neural crest, &c., to the aorta, and, *secondly*, from the aorta to near the spinal ganglion.

The reasons for this complicated migratory movement have yet to be discovered, but the evidence for it is, we think, sufficiently definite. The situation of the definitive sympathetic in the vertebral canal in birds was pointed out as long ago as

1810 by Tiedemann, but the occurrence of a primary chain in a position analogous to that of the definitive sympathetic in other vertebrates was not discovered till many years later by His. Subsequent writers have all supported his findings, but hitherto all have failed to discover any genetic continuity between the two formations. Ganfini (**5**), in his paper on the sympathetic in birds, seems to have observed the relations more successfully than his predecessors, but he failed to discover the origin of the secondary ganglia from the dorso-lateral processes of the primary ganglia. He follows his predecessors in deriving them immediately from the dorsal root ganglia, for he writes: "Alla formazione del cordone simpatico primario segue la formazione di un cordone simpatico secondario. Questo è formato da una serie di gangli situati ognuno a livello della parte del tutto prossimale del nervo spinale ed originati da neurociti derivanti esclusivamente dai gangli spinali. . . . Da ogni ganglio simpatico secondario si diparte un ramo che dirigendosi ventralmente e medialmente connette il cordone simpatico secondario col primario." From the last statement he appears to have observed connections proceeding *from* the secondary *to* the primary ganglia, while our graphic reconstructions show exactly the opposite.

Text-figure 20.



Transverse section of Sparrow 69 (= 8-day chick), showing the differentiation of cells of the dorsal root ganglion (*D.R.G.*) and of the secondary sympathetic ganglion (*Sy.G.*). *Sp.N.* Spinal nerve.

The nearest approach to correct observation seems to have been made by Miss Williamina Abel (**1**), though curiously enough she attached little importance to it. In her paper we read:

"At 144 hours the permanent ganglia appear as small clusters of nerve cells, lying close to the mixed spinal nerves and connected to them by fibrous *rami communicantes.* These ganglia increase rapidly in size and are formed largely from the sympathetic cells which have accumulated between the spinal nerves and the temporary (*i.e.* primary) sympathetic chain. Some of the cells of the temporary sympathetic chain are also incorporated in the permanent (*i.e.* secondary) ganglia. Besides these the permanent ganglia are formed from the cells which have migrated directly to them along the spinal nerves. That portion of the temporary sympathetic chain which is not incorporated in the ganglia of the permanent chain gradually atrophies and disappears."

Our observations therefore agree with Miss Abel's in so far as they confirm the importance of the contribution from the primary to the secondary chain. They differ, in assigning a definite form to the accumulations of which she speaks, and in demonstrating definite paths by which this contribution is effected, *i.e.* the dorso-lateral prolongations from the primary ganglia. Moreover, from the marked differentiation of the spinal nerve cells from those of the sympathetic ganglia already described, we question the existence of small clusters of nerve cells lying close to the mixed spinal nerve, in so far as she derives them from the spinal nerve directly. At the time when such clusters do occur they will be found to consist of small cells with dark nuclei containing many granules. We cannot see how such a metamorphosis should occur in the short passage from the dorsal ganglia, all the cells of which are large, with pale nuclei and containing many granules. It seems more probable that the whole definitive sympathetic ganglion arises from the primary sympathetic chains by migratory cells, and by active mitosis, which latter can always be observed in these ganglia.

As to the fate of the primary sympathetic, Miss Abel is in accord with previous writers, all of whom express the opinion that in the course of further development it atrophies and disappears. Our material does not attain a sufficiently advanced stage for us to express a definite opinion on this question, but there are clear evidences of its presence in the oldest stage we have observed, Sparrow 69 (= 8-day chick). The definitive sympathetic chain in the cervical region of this embryo runs in the vertebrarterial canal. Between the vertebræ of the hinder part of the neck, small bundles of fibres emerge and run ventrally, but closely apposed to the cartilaginous vertebral column; these fibres enter large and distinct medial ganglia, lying just under the vertebral column, and above the carotids. In Sparrow B, a little younger, three such medial ganglia are specially noticeable (see text-fig. 11). Longitudinal fibres run from these ganglia to the tissues lying between them, which are highly vascular, being traversed by a capillary plexus linked up with the carotids. The ultimate destiny of these ganglia awaits further investigation.

Apart from them the primary cords are so reduced, by the migration above described, that they may be considered to be wholly absorbed by the definitive sympathetic nervous system. It should be stated that the primary sympathetic chain contributes also to the formation of cœliac and other ganglia, thus the degeneration of the primary sympathetic chains, described by previous writers, should rather be termed a migration from the primary location.

We are unable to assign a cause for this migration of the primary sympathetic chains from the dorso-lateral angles of the aorta to their definitive position, as secondary sympathetic chains in the vertebrarterial canal. Thébault, in his description of the sympathetic system in adult birds, suggests that the long thin neck of the bird renders the sympathetic liable to lesions; hence he explains its inclusion in the vertebrarterial canal. If this be the case, we may regard the definitive location as an adaptive modification. It is perhaps worth notice that the reptile in which primary and secondary sympathetic chains have also been described, viz. the tortoise, is also endowed with a long and flexible neck.

In conclusion, we would express our gratitude to Professor J. P. Hill, F.R.S., for his generosity in supplying us with such an abundance of excellent material and for much assistance in the course of our work and in the preparation of this paper. We are indebted to Professor D. M. S. Watson, F.R.S., for friendly criticism and for aid in final preparation for the press.

I. *Summary.*

1. The primary sympathetic primordia originate from the spinal cord, and are hence ectodermal in origin.
2. The primordia of the ganglia of the primary sympathetic chain are laid down metamerically at inter-somitic points.
3. The constituent cells of the primordia are derived from
    - *a.* The neural crest;
    - *b.* The dorsal root ganglia;
    - *c.* The neural tube by way of the ventral root.
4. The primordia extend cranially and caudally to form a continuous chain on either side.
5. The primary ganglionic swellings extend dorso-laterally. The free ends of these extensions enlarge and becoming separated from the primary ganglia give origin to the definitive sympathetic ganglia.
6. As in the case of the primary ganglia these definitive ganglia extend cranio-caudally and unite to form a continuous chain.
7. The primary sympathetic gives origin to
    - *a.* The secondary sympathetic chains;
    - *b.* The chromaffin cells of the medulla of the suprarenal body;
    - *c.* The cœliac and other ganglia;
    - *d.* The unpaired ganglia in the cervical region.

K. *Bibliography.*

A very full bibliography of the development of the sympathetic nervous system up to the year 1905 will be found in Hertwig's 'Handbuch der vergleichenden und experimentellen Entwickelungslehre,' Band ii. Teil iii.

More recent contributions to the literature are given by Goormaghtigh and also by Da Costa in the papers cited below.

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